Abstracts of Papers

Twenty-seventh Annual Albert L. Tester Memorial Symposium, 21–22 March 2002[1]

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert L. Tester, who, at the time of his death in 1974, was senior professor of zoology at the University of Hawai'i at Mānoa. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields within marine biology. Papers reporting original research on any aspect of science are solicited from students at the university and these papers are presented at the symposium, which takes place during the spring semester. Income from contributions to the Albert L. Tester Memorial Fund of the University of Hawai'i Foundation is used to provide prizes for the two best papers, judged on quality, originality, and importance of research reported, as well as the quality of the public presentation. The Waikīkī Aquarium presents the Mike Weekley Award, based on the same criteria. Judges include Department of Zoology faculty members and the previous year's student award winners. In addition, a distinguished scholar from another university or research institution is invited to participate in the symposium as a judge and to present the major symposium address. This year the guest participant was Dr. Marc Mangel, Professor of Environmental Studies at the University of California at Santa Cruz.

Hot Water Drench Treatments for Control of Burrowing Nematode in Rhapis and Fishtail Palms

Albert C. Arcinas[2]

Exporters of potted nursery stock face strict quarantine regulations against the burrowing nematode (BN). Currently, there are no approved quarantine treatments to disinfest plants of BN. Interceptions lead to substantial economic loss and curtailment of trade. Therefore, hot water drench treatments were investigated for quarantine utility. Drenches with 50°C water were applied for 10–16 min to two economically important palm species, rhapis and fishtail. Each plant was inoculated with 5000 mixed life stages of BN and allowed to establish for 14 weeks before treatment. In rhapis palms, a moderately good host, a 16-min hot water drench had the highest efficacy, achieving 99.6% mortality of BN. In fishtail palms, a poor host, all treatments longer than 10 min at 50°C achieved 100% mortality. Probit regression estimates were used to estimate LT_{99}, resulting in 16.9 and 10.3 min, respectively; however a χ^2 goodness-of-fit test for deviation from observed data was significant for rhapis palms. The high efficacy of hot water drenches for the control of BN is approaching the Probit 9 standard of 99.9968% mortality that is required for U.S. Department of Agriculture approval as a quarantine treatment.

[1] Manuscript accepted 1 May 2002.

[2] Department of Plant and Environmental Protection Sciences, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Brent S. Sipes.

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Evolving Morphology of an Open-Channel Lava Flow on Mt. Etna

John E. Bailey[3]

From mid-May to mid-July 2001 a small eruption from the southeast cone on Mt. Etna into the Valle del Leone persistently fed a classic example of a compound flow field, with multiple bifurcated channels that extended 1 km across and 2 km down the mountain. This flow field provided the opportunity to study changes in thermal structure and morphology of an open lava channel, over a period of days. This was done using temperature-calibrated digital images from a FLIR (forward-looking infrared) camera and continuous recordings from a radiometer, which provided an integrated radiance value over the area of the channel in the field of view. The images and patterns of change in these data showed many features, such as different types of tube formation, channel blockages, overspills, diverted flows, crust formation/breakup, and "surges" in the volume of lava flowing in the channel. There were small surges or "pulses" and large surges that totally overfilled the channel. Pulses resulted from lava buildup behind channel blockages, which then partly broke up, allowing rapid lowering of the channel fill level, before blockages formed again. The larger surge events, however, completely overwhelmed the channel and were attributed to a change in supply volume. Changes in integrated thermal output (of the channel) lag behind when compared with changes in surface velocity of flowing lava, which is consistent with the hypothesis of a changing supply volume. This study represents one of the first uses of a FLIR to study active lava flows and suggests that it has great potential for helping understand eruption dynamics.

Size Differences Influence Aggressive Behavior in the Mangrove Blenny, *Omobranchus rotundiceps obliquus*

Erin Baumgartner[4]

The mangrove blenny, *Omobranchus rotundiceps obliquus*, is an invasive species found in sheltered fouling communities in Kāne'ohe Bay, O'ahu. The fish arrange their territories in a diffuse fashion, where the territory is a small area around the refuge. The territory moves along with the fish as it switches refuges. Access to these refuges is controlled by position in the dominance hierarchy. Aggression and aggressive interactions influence the position of the fish in the dominance hierarchy. This study examined those factors that influence the likelihood of aggressive behavior occurring between individual blennies. Two laboratory experiments involving communities and dyads of blennies examined the influence of size, sex, and size asymmetry on aggressive behavior. Size and sex did not have a significant influence, but size asymmetry significantly influenced aggressive behavior in blennies. The proportion of aggressive encounters, ratio of one-sidedness of the relationship, and number of fights between pairs in communities of blennies were all significantly influenced by size asymmetry, with similar-sized combatants being more likely to behave aggressively to one another. The number of high-intensity interactions and amount of time spent on high-level intensity interactions were significantly influenced by size asymmetry in dyad trials of blennies. The amount of time it took to achieve a dominance relationship as indicated by a color shift was also significantly influenced by size

[3] Department of Geology and Geophysics, Hawai'i Institute of Geophysics and Planetology, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Andrew Harris.

[4] Department of Zoology, and Hawai'i Institute of Marine Biology, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Dave Greenfield.

asymmetry. Blennies that were similar in size spent more time behaving aggressively, had more aggressive interactions, and took longer to achieve a dominance relationship.

Acoustic Backscattering Properties of Hawaiian Lutjanid Snappers

Kelly Benoit-Bird[5]

Acoustic properties and swim bladders of six species of commercially important bottom-dwelling Hawaiian lutjanid snappers, for which backscatter data are not available in the literature, were investigated using broadband sonar, X rays, and plaster swim bladder casts. X rays revealed species-specific differences in shape, size, and orientation of the swim bladders, shown to be the primary source of backscattering. Backscatter data were obtained from anesthetized, live fish mounted on a rotor and rotated around each of their three axes. Fish were ensonified with broadband signals (60–200 kHz). As in other fish species, maximum dorsal aspect target strength was correlated with fish length within each species. In situ echoes taken from a submersible were not significantly different from those from surface measures, showing that surface measures are valid for fish at depth. Species-specific swim bladder characteristics were correlated with changes in echo characteristics. For example, the angle between the fish's dorsal aspect and the major axis of its swim bladder was consistent between individuals within a species. This angle had a one-to-one relationship with the angle at which the maximum dorsal aspect target strength was measured. Species-specific differences in echo structure characteristics such as number of highlights and length of the echo were also evident, as were differences in spectral characteristics such as peak frequency and degree of spectral rippling. Distributions of these characteristics did overlap between species; however, with a combination of multiple broadband acoustic characteristics, species identification is possible.

Pattern in Hawaiian Rocky Intertidal Community Structure along Wave Exposure Gradients

Christopher E. Bird[6]

Community structure on Hawai'i's rocky intertidal shores has received relatively little attention when compared with reef, pelagic, forest, and grassland communities. In fact, there is a common misconception that there is no intertidal in Hawai'i due to the small 0.85-m (2.8-ft) tidal fluctuation. I deployed maximum wave force dynamometers, temperature loggers, and wetted sponges on transects running perpendicular to the shoreline along a wave exposure gradient. Wave force was correlated with lower temperature and desiccation at a given height above mean lower low water. I propose that this is due to wave height approaching and/or eclipsing the tidal range, effectively expanding the zone of biotically favorable habitat for intertidal organisms above that set by the tides. Thus physical conditions on the shore grade from tide domination in low wave exposure to wave domination in high wave exposure. I successfully used digital photography to sample the rocky intertidal community along wave exposure gradients on O'ahu and Maui. Turf and macro algae were much more prominent in

[5] Department of Zoology, and Hawai'i Institute of Marine Biology, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Whitlow Au.

[6] Department of Botany, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Celia Smith.

mid- to low wave exposures than in high wave exposure. Crustose algae and macro grazers such as *Colobocentrotus atratus* were most abundant in zones receiving high wave exposure. Gross community structure on Hawaiian rocky intertidal shores is correlated with physical conditions controlled by wave exposure.

Integrated Management Strategies against *Aspidiotus destructor* (Homoptera: Diaspididae) in Banana

Ming-yi Chou[7]

Infestations of *Aspidiotus destructor* Sign. in banana cause substantial loss for Hawai'i's growers at the export market. Integrated pest management tactics were evaluated against the quarantine pest. Seasonal population dynamics were investigated at six commercial orchards to analyze population density and spatial distribution of *A. destructor* in banana. No significant difference was found between seasonal population densities at most survey locations. Low population densities were observed, with maximum density at 1.38 on a 5-point density scale. Formulated pesticides diazinon (Diazinon), imidacloprid (Provado), pyriproxyfen (Esteem), and thiamethoxam (Actara) were found to be effective against nymphal stage, and adult mortalities varied from 0.39 to 0.96. Complete control of adults was achieved by hot water immersion with temperatures ranging from 47 to 49°C.

Nearshore Nursery Use in the Scalloped Hammerhead Shark, *Sphyrna lewini*

Kanesa Duncan[8]

Scalloped hammerhead shark pups (*Sphyrna lewini*) inhabit nursery grounds in coastal embayments where they act as apex predators and may seasonally dominate vertebrate biomass. I tested several hypotheses concerning pups' distribution, residency, growth, and survivorship in nursery areas by conducting mark-recapture and captive growth studies of young *S. lewini* in Kāne'ohe Bay, O'ahu, Hawai'i. Shark pups were present in the bay during all months of the year, with highest catch rates in July, August, and September. I found little spatial segregation of pups within Kāne'ohe Bay and although the majority of recaptures occurred close to the point of release, some sharks dispersed up to 5 km across the bay after only 1 week at liberty. These data are contrary to previous suggestions that pups congregate in the south part of Kāne'ohe Bay. Most recaptures occurred within 4 months of tagging, but some sharks were at liberty for more than 1 yr. Combined growth rate data from recaptures and from captive sharks suggest that *S. lewini* pups grow very slowly in the months immediately after birth. Presence of larger juvenile sharks within the bay, recapture of sharks at liberty for over a year, and growth rates of captive sharks suggest that coastal nursery areas may remain important hammerhead habitat throughout the first 3 yr of life. This finding has important fishery implications for Kāne'ohe Bay and also for pupping grounds throughout tropical waters where *S. lewini* is heavily gillnetted.

[7] Department of Plant and Environmental Protection Sciences, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Ronald F. Mau.

[8] Department of Zoology and Hawai'i Institute of Marine Biology, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Kim Holland.

Role of the Gene *Distal-less* in Development of *Euprymna scolopes*

Claudia Farfán[9]

Distal-less encodes a homeodomain protein best known for its role in proximo-distal patterning of *Drosophila* limbs. Expression surveys of presumptive *Distal-less* (*Dll*) orthologues in coelomate phyla, including chordates, has prompted the suggestion that this gene is ancient and may be involved in patterning of body wall outgrowths of protostomes and deuterostomes. However, these surveys did not include representatives of the Mollusca, a phylum characterized by morphological plasticity and unique anatomical specializations. Cephalopod arms are conspicuous body wall outgrowths, appendages nonhomologous to other structures outside the Mollusca, and a morphological novelty within the taxa. Thus it is of interest to know if *Dll* function is required for formation of these appendages. In a first phase to assess the role of *Dll* in development of the arms of the sepiolid *Euprymna scolopes*, I isolated a fragment of the *Dll* homeobox by RT-PCR and performed a preliminary expression study (whole mount) using a highly cross-reactive *Dll* antibody raised against the butterfly *Precis coenia Dll* homeodomain. The isolated fragment (66 base pairs long) is 95% homologous to the *Dll* of *P. coenia* and *Tribolium castaneum*, and to a number of vertebrate *Dlx* orthologues. The presumptive *E. scolopes Dll* is expressed in the arms, funnel, eyes, optic lobes, and all over the epidermis. These data suggest that *Dll* has a pleiotropic role in development of *E. scolopes*, including arm development.

Genetic Population Structure in Relation to Dispersal Potential in Pacific Nudibranchs

Anuschka Faucci[10]

Marine organisms vary greatly in their potential for dispersal of larvae. Levels of gene flow among populations depend on this potential and the extent to which it is realized. Population genetic data can be used as an indirect measurement of dispersal in marine species. Species of the nudibranch genus *Phestilla* occur throughout the Pacific, feeding on different coral species. They differ in their larval developmental mode and therefore dispersal potential (e.g., *Phestilla sibogae* and *P. minor* have lecithotrophic larvae [nonfeeding, short planktonic period], whereas *P. lugubris* has planktotrophic larvae [feeding, obligate long planktonic period]). A 680–base pair region of the mitochondrial cytochrome *c oxidase I* (*COI*) gene was sequenced for the following species: *P. sibogae* (O'ahu and Guam), *P. lugubris* (Guam), *P. minor* (O'ahu and Guam), *P. melanobrachia* (O'ahu and Guam), *Phestilla* sp. 1 (Guam), *Phestilla* sp. 2 (Guam). A neighbor-joining tree was produced using the two nudibranchs *Caloria indica* and *Hypselodoris infucata* as outgroups. *P. sibogae* from Guam and *P. lugubris* from Guam are genetically more closely related than *P. sibogae* from Guam and O'ahu. *Phestilla sibogae* from O'ahu has an intrapopulation divergence of 0–0.3% (mean 0.19%), *P. sibogae* from Guam 0–0.6% (0.4%), and *P. lugubris* from Guam 0–0.6% (0.4%). These preliminary results indicate no substantial intrapopulation structuring, although no interpopulation values have yet been obtained. Expected results would have been that *P. lugubris* has a lower intraspecific divergence than *P. sibogae*. All other species are genetically distinct from each other and show some degree of geographical structuring.

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[9] Department of Zoology, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: H. G. de Couet.

[10] Department of Zoology, and Kewalo Marine Laboratory, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Michael G. Hadfield.

Top-Down and Bottom-Up Influences in a Hawaiian Arboreal Arthropod Food Web[11]

Daniel S. Gruner[12]

Predator (top-down) and resource (bottom-up) influences in food webs are strong and pervasive, but few studies have investigated their interactive effects in real terrestrial ecosystems. This study focused on arthropods associated with the dominant species in these young successional systems, *Metrosideros polymorpha* (Myrtaceae), also the dominant tree in the Hawaiian Islands. In August and September 1998 on a 120-yr-old flow, severe nutrient limitation was removed by fertilization and combined with bird predator removal cages in a large-scale, well-replicated, randomized block design. Arthropod densities were measured from foliage clipping samples at the outset and conclusion of the experiment. After 2.75 yr, foliar nitrogen content and *M. polymorpha* growth were directly increased in fertilized relative to unfertilized plots, but were unaffected by indirect effects of top predator exclusion. Fertilization increased densities of detritivores, and bird exclusion increased spider densities. Arthropod combined densities, besides spiders, were reduced in the interaction treatment, suggesting cascading effects of these intermediate-level predators to the detritivores. Herbivore numbers were unchanged by either treatment, but herbivores were sensitive to another bottom-up influence, foliar pubescence, as confirmed by separate arthropod collections. Top-down effects in this system are complementary, not purely additive, to bottom-up influences, and details are dependent on structure of the food web.

Scattering of the Oceanic Internal Tide from a Seamount

Shaun Johnston[13]

Internal tides are generated as the surface tide forces stratified water up and down the flanks of midocean topography. Internal tides are generated on length scales ranging up to 150 km. Smaller-scale waves are dissipated near the topography, contributing to mixing of the deep ocean. Larger-scale waves are observed to propagate away from the topography, with decay scales on the order of 1000 km. These waves carry much of the energy contained in the oceanic internal wave field, and it is not well known how they are dissipated. So the question addressed here is: how is energy transferred from the large-scale internal tides to smaller scales and ultimately to the smallest scales where mixing takes place? In a numerical model, we produced a large-scale internal wave on the western boundary and let it propagate 5 wavelengths before encountering an idealized Gaussian seamount in a constant stratification ocean. We found greatly enhanced energy density directly over the crest of the seamount and a strong directional distribution (i.e., regions of enhanced and reduced energy density) in the lee of the seamount. Over the seamount the sloping topography forced the incident internal tide to smaller horizontal and vertical length scales, thereby increasing current shear. These sheared currents were dynamically unstable and led to enhanced mixing directly over the topography.

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[12] Department of Zoology, and Ecology, Evolution, and Conservation Biology program, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Andrew Taylor.

[13] Department of Oceanography, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Mark Merrifield.

Investigating Symbiont-Induced Hemocyte Trafficking during Light Organ Morphogenesis in Hawaiian Bobtail Squid, *Euprymna scolopes*

Tanya Koropatnick[14]

The symbiotic association between the sepiolid squid *Euprymna scolopes* and the luminous marine bacterium *Vibrio fischeri* begins when the hatchling host gathers symbionts from the environment using currents created by two ciliated epithelial fields on the surface of the host light organ. Upon infection with *V. fischeri*, the light organ undergoes a 4-day morphogenesis involving widespread apoptosis in the ciliated epithelia, and regression of the ciliated fields, including the anterior and posterior ciliated appendages. This study investigated the potential role of host hemocytes in this dramatic morphogenesis. Confocal microscopy was used to characterize hemocyte trafficking within the blood sinuses of the ciliated appendages over time in both symbiotic (infected with *V. fischeri*) and aposymbiotic (uninfected) animals, and in animals exposed to microbial products, lipopolysaccharide (LPS), lipid A, and peptidoglycan (PG). In the presence of the symbiont a significant increase in hemocyte number could be seen in the sinuses as early as 2 hr postinfection, and a peak in number was reached by 24 hr. A significant increase in hemocyte number was also induced by both LPS and PG alone or in combination. LPS has been shown to induce cell death in the ciliated epithelium, but PG alone did not induce apoptosis. Lithium chloride, which delays hemocyte migration, decreased symbiont-induced hemocyte trafficking while cell death levels remained unaltered. These data suggest that hemocytes are not mediating apoptosis during light organ morphogenesis. However, high numbers of hemocytes continue to be present in the blood sinuses throughout regression, suggesting a role for these cells in the loss of the epithelial appendages.

Hox Genes and the Cephalopod Arm Crown: Homology and Morphology[15]

Patricia N. K. L. Lee[16]

Cephalopods are highly derived mollusks. One striking evolutionary modification is the derivation of a prehensile arm crown from the molluscan foot. Arms are an important morphological character used in cephalopod taxonomy, dividing coleoid cephalopods into Octopodiformes (octopods with four arm pairs) and Decapodiformes (squids with five arm pairs). The ancestral coleoid is proposed to have possessed five pairs of unmodified arms. Which arm pair has been lost in the octopods? Relying solely on morphological comparisons, assigning arm homologies between the two groups and determining which arm pair has been lost have been difficult. In addition, there have been morphological specializations of specific arms in different groups. How might these specializations have evolved? To address these questions, we examined *Hox* genes in the sepiolid squid *Euprymna scolopes* as potential molecular markers. *Hox* genes are key regulatory genes involved in specifying body regions along the anterior-posterior axis of all bilaterians. We determined the expression patterns of eight *Hox* orthologues during *E. scolopes* development by whole-mount in situ hybridization. Each arm pair expresses a unique combination of *Hox* genes during development, which

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[14] Department of Zoology, and Pacific Biomedical Research Center, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Margaret McFall-Ngai.

[15] Supported by Sigma Xi GIAR, Hawaiian Malacological Society, A&S Research Advisory Council, and Edmondson GIAR.

[16] Department of Zoology, and Kewalo Marine Laboratory, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: H. G. de Couet.

may play a role in specifying arm identity. Although this is the first cephalopod in which these genes have been studied, we suggest that *Hox* genes may potentially be used as molecular markers for verifying arm homologies between octopods and decapods. In addition, with a potential role in body region specification, *Hox* genes may be involved in modifications of various arms during cephalopod evolution.

Molecular Investigations into the Phylogenetic Position of the Chaetognaths

David Matus[17]

Chaetognaths, or arrowworms, are small marine invertebrates, ubiquitous in the zooplankton in all the world's oceans. For centuries, the position of this phylum within Metazoa has been enigmatic at best, having been allied with a wide variety of other phyla, due to a variety of confusing morphological and developmental characters. Although most invertebrate textbooks place the chaetognaths within the deuterostomes, molecular evidence from the last eight years suggests that this placement is inaccurate, though no clear consensus exists in the literature as to their true affiliation within Metazoa. Recent work involving a "total evidence" approach combining morphological, developmental, and molecular data suggests an affiliation of chaetognaths to ecdysozoans, a clade of molting animals within the protostomes. Previous studies have attempted to resolve their position using structural and housekeeping genes, but this work represents the first use of developmental regulatory genes (genes that code for body plan formation, axial patterning, etc.) to determine the position of the chaetognaths within Metazoa. A PCR-based survey of a local species of chaetognath, *Flaccisagitta enflata*, has resulted in the identification of several different homeobox genes, including three characteristic ecdysozoan *Hox* gene orthologues: *Ultrabithorax*, *Abdominal B*, and *Antennapedia*. Multiple methods of phylogenetic analysis suggest that the *Antennapedia* orthologue is more closely related to other ecdysozoan *Antennapedia* orthologues than to any other group of genes, providing support for the inclusion of the chaetognaths within Ecdysozoa.

Drosophila Lysyl Oxidases: Effect of *Dmloxl-1* on Chromatin Structure and Transcription and Adult-Specific Expression of *Dmloxl-2*

Janos Molnar[18]

Lysyl oxidase (LOX) is a copper-containing amine oxidase best known for its role in catalyzing cross-linking in elastin and fibrillar collagens. In addition to its extracellular functions, its intracellular, intranuclear locations, and involvement in tumor suppression and senescence reflect a remarkable functional diversity of LOX. The existence of multiple LOX-like proteins in mammals, however, makes it difficult to interpret how LOX, or the LOX-like proteins, may individually contribute to these diverse functions. We have identified two LOX-like genes, *Dmloxl-1* and *Dmloxl-2*, in *Drosophila melanogaster*. The temporal division of gene activities allowed functional analysis of *Dmloxl-1* alone in early development. Selective inhibition of *Dmloxl-1* by β-aminopropionitrile (β-APN) resulted in developmental delay, altered pupation, a shift in sex ratio

[17] Department of Zoology, and Kewalo Marine Laboratory, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Mark Q. Martindale.

[18] Department of Anatomy, Pacific Biomedical Research Center, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Katalin Csiszar.

toward fewer males, enhanced position effect variegation (PEV) in males, and lower mRNA levels of seven ribosomal and the glue protein genes. Transgenic *Drosophila* females, but not males, overexpressing *Dmloxl-1* demonstrated suppression of PEV. These results suggest that *Dmloxl-1* functions as a trans-acting regulator of transcription rate acting through compositional changes of the chromatin.

More than Just a Pretty Pattern: What the Spots on the Shell of *Cribrarula cribraria* (Mollusca: Cypraeidae) Tell Us about Its Anatomy

Fabio Moretzsohn[19]

The mantle of cowries is stationary in relation to the shell, meaning that the mantle always returns to the same region of the shell. When fully exposed, the mantle covers the whole shell, and it is the mantle that deposits the $CaCO_3$ layers that compose the shell. In the *Cribrarula cribraria* complex, the characteristic white dorsal spots (DS) on the shell are formed by nonpigmented oval "windows" through which the white background of the dorsum is seen. It is hypothesized here, based on direct observation and photographs of live cowries, that there is a direct correspondence between DS and mantle papillae in the *C. cribraria* complex, with the white DS being the record of each papilla at the time the pigmented layer was laid. DS counts from 371 shells assumed to represent all 10 species in the *C. cribraria* complex show species-specific ranges of DS counts. Other DS-related useful taxonomic characters include position of the dorsal line (indicating relative sizes of each of the mantle lobes that cover the shell); number of dorsal papillae on each mantle lobe; and relative size, density, and sharpness of DS. The results obtained in the study of the *C. cribraria* complex can be extrapolated to other cowries with similar dorsal patterns, such as *Lyncina leucodon* and *Mauritia mauritiana*. Cowrie shells are considered to have few informative characters, with the glossy, involute shell bearing little sculpture (except for the apertural teeth), spines, and not even the protoconch showing. Combined with other data such as anatomical and molecular characters, the nontraditional conchological characters used here may provide a higher resolution than traditional conchological characters needed to solve some of the taxonomic problems in cowries.

Use of DNA Arrays for Molecular Systematic Studies of Species within the *Bactrocera dorsalis* Complex[20]

Chrystie Naeole[21]

The *Bactrocera dorsalis* complex is a group of more than 50 closely related species. A number of these species are major economic pests. Current taxonomic keys are deemed inadequate to make accurate species identification, and little is known about the systematic relationships of species within this complex. DNA markers provide characters that may be useful for both purposes. Using PCR primers designed from conserved coding regions of specific genes, we have amplified intron regions of these genes for use as genetic markers. Introns are useful because they tend to harbor differences to a greater extent compared with coding regions and genes. We are working to demonstrate how

[19] Department of Zoology, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: E. Alison Kay.

[20] Supported by CDFA Grant No. 97-0451.

[21] Department of Cell and Molecular Biology, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: David Haymer.

DNA sequences from intron regions of particular genes can be combined with DNA array technology to identify and distinguish species within this complex. This approach may also help in characterizing new and undescribed species known to occur within this complex and help in the resolution of the systematic relationships of these species.

Propagation of Breadfruit for Conservation and Germ Plasm Exchange

Cynthia Nazario[22]

The National Tropical Botanical Garden (NTBG) has established a breadfruit collection at the Kahanu Garden in Hāna, Maui. The collection consists of 173 accessions from 17 Pacific Island groups, Indonesia, the Philippines, and the Seychelles. From this collection a core group of breadfruit cultivars has been selected in which further in-depth research is being conducted, including tissue culture, air-layering, and seed germination. Breadfruit is currently propagated vegetatively through root cuttings or shoots. These methods, although successful, are slow and can make transport of plant material over long distances difficult. Currently, I am developing a methodology for tissue culture of breadfruit as a method of germ plasm exchange. This methodology includes selection of an explant source, development of a sterilization protocol, initiation and proliferation of shoots on various in vitro media, rooting of shoots in vitro, and hardening of plantlets. In addition to in vitro propagation, breadfruit air-layer studies comparing auxin concentrations, etiolation, branch size, and seasons are also being conducted to provide another propagation alternative. Both these studies will facilitate the distribution of breadfruit for propagation and research throughout the world while assisting in conservation efforts of endangered breadfruit varieties.

Role of Pre-B Cell–Colony Enhancing Factor (PBEF) in Human Fetal Membranes[23]

Simona Ognjanovic[24]

Pre-B cell colony–enhancing factor (PBEF) is a novel cytokine, which is constitutively expressed in human fetal membranes throughout pregnancy. It was shown in our laboratory that this is a mechanically responsive gene upregulated in the fetal membranes both in vitro by their distension and in vivo by the process of labor. In addition, infection of this tissue substantially increased PBEF expression. We hypothesized that a mechanically induced protein might cause growth of the tissue to reduce mechanical strain. Thus, recombinant human PBEF (rhPBEF) was produced in a bacterial system and added to amniotic epithelial (WISH) cells at 1, 10, and 100 ng/ml for 4 days. Proliferation was assayed with the CellTiter 96 Proliferation Assay (Promega). rhPBEF (100 ng/ml) caused a significant increase in their proliferation when compared with the control (incubated with media only). IGF-II (30 ng/ml) was used as a positive control. To confirm and extend these results, fetal membrane explants from three different patients with no histological evidence of infection were treated with rhPBEF (100 ng/ml) for 4 hr; controls were adjacent sections incubated in culture

[22] Department of Tropical Plant and Soil Science, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Diane Ragone.

[23] Supported by NIH grant HD24314.

[24] Department of Cell and Molecular Biology, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Gillian Bryant-Greenwood.

media only. Total RNA was extracted and the expression of 847 genes was compared on the human cytokine cDNA expression arrays. Results show that PBEF causes increased expression of several genes involved either in growth regulation or in infection-induced pathways. These data are being confirmed by Northern blotting.

Development of *Anagyrus ananatis* (Hymenoptera: Encyrtidae) at Constant Temperatures

Raju Pandey[25]

Pink pineapple mealybug, *Dysmicoccus brevipes* (Homoptera: Pseudococcidae), is one of the most widely distributed mealybugs around the world. In association with pineapple mealybug wilt–associated closterovirus, *D. brevipes* can lead to the expression of mealybug wilt disease. *Anagyrus ananatis* Gahan, a solitary endoparasitoid, is the most commonly distributed biological control agent of *D. brevipes* in Hawaiian pineapple fields. Two experiments were conducted to determine effects of constant temperature on development of *A. ananatis*. In the first experiment, parasitized *D. brevipes* was incubated at five different constant temperatures (T), 14.6°C, 19°C, 23.8°C, 28.9°C, and 31°C. Time (D) taken to complete the life cycle (from oviposition to adult emergence) was noted. A new variable, DT, was calculated and analyzed by regression analysis of DT with T. The lower temperature threshold (T_o) was determined to be 12.68°C with a total heat requirement of 271 DD above T_o for *A. ananatis* to complete its life cycle. A typical pineapple plantation accumulated 4311 DD of heat above 12.68°C annually, allowing *A. ananatis* to complete about 16 generations per year. In the second experiment, parasitized *D. brevipes* was incubated at 23.8°C and 20 mealybugs dissected at 24-hr intervals to determine the life cycle of *A. ananatis*. Results showed that eggs began to eclose after 2 days of oviposition and completed hatching in 4 days. Larvae were observed from 2 days after oviposition until 9 days after oviposition, the prepupa stage was observed from 8 to 12 days, and the pupa stage from 10 to 25 days after oviposition. Adult emergence began after 24 days of oviposition and was completed in 26 days. These findings are useful in the development of mass rearing techniques for *A. ananatis* for augmentative biological control of *D. brevipes*.

Trophic Ecology of Two Oceanic Squids in Hawaiian Waters

Matthew Parry[26]

The two study species are important members of the pelagic ecosystem. *Sthenoteuthis oualaniensis* (purple squid) has been the target of a traditional local fishery for decades, and *Ommastrephes bartramii* (red squid) was once the target of a worldwide fishery. Understanding the trophic dynamics of these squids is vital to understanding the pelagic ecosystem as a whole. Stomach contents and stable isotope analyses using several tissue types were used to determine the trophic status and trophic interactions of these squids. Stomach contents analyzed (*O. bartramii* [$n = 264$] and *S. oualaniensis* [$n = 210$]) indicated that both squids feed heavily on myctophids, with the purple squid feeding almost exclusively (≅80%) on myctophids and the

[25] Department of Plant and Environmental Protection Sciences, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Marshall W. Johnson.

[26] Department of Oceanography, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Richard Young.

red squid appearing to feed more generally on several families of fishes. The main myctophid prey is *Symbolophorus evermanni* in both species of squid predators. Stable isotopes show that the red squid occupies a position roughly 1.5 trophic levels above the purple squid. The red squid appears to range about three trophic levels throughout its life from paralarvae to adult; the purple squid ranges only about two trophic levels over its life span. The red squid seems to plateau at its highest trophic level at mantle lengths of over 300 mm, but the purple squid does not appear to plateau throughout the size ranges studied. Using the stable isotope signatures of blood and secreted hard structures such as the eye lenses gives good agreement with the values and trends seen in isotope values taken from the mantle tissue of various sizes of squids.

Plate Tectonics in the Southwest Pacific: GPS Estimates of Crustal Velocity in the Tonga-Lau System

David A. Phillips[27]

The Tonga-Lau system is remarkable for having the highest rates of subduction and back-arc spreading on Earth, the highest levels of deep mantle seismicity, and some of the most pronounced mantle velocity and Q anomalies observed on Earth. Bevis et al. provided initial crustal velocity estimates for this system based on Global Positioning System (GPS) survey campaigns conducted in 1990 and 1992. In 1996, I helped establish a continuous GPS (CGPS) network throughout the region and have reoccupied the original survey markers several times between 1996 and 2000. I have reanalyzed the entire 1990–2000 time series using GAMIT/GLOBK geodetic software. Convergence estimates between the Pacific Plate and the Tonga Ridge range from 150 to more than 240 mm/yr in a Pacific-fixed reference frame. These estimates are far more accurate than those reported previously and represent the fastest tectonic velocities ever measured. Differential motion within the Tonga Arc was observed for the first time, with Niuatoputapu Island moving northeastward relative to other sites on the Tonga Ridge. This differential motion may suggest the presence of a previously unknown microplate. Counterclockwise rotation of the Fiji Platform was also observed for the first time in an Australia-fixed reference frame. Formal error estimates are at the mm-level and are supported by comparisons between velocities determined at nearly coincident campaign and CGPS stations in several islands. This work contributes to a greater understanding of the current plate tectonic regime in the Southwest Pacific and provides insight into the development and behavior of major plate/microplate interactions throughout the world and throughout Earth's history.

(References: Bevis, M. G., F. W. Taylor, B. E. Schutz, J. Recy, B. L. Isacks, S. Helu, R. Singh, E. Kendrick, J. Stowell, B. Taylor, and S. Calmant, *Nature* **375**, 249–251 (1995).)

Grazing Impact of *Oikopleura fusiformis* on Picoplankton in the Coastal Subtropical Pacific

Rebecca D. Scheinberg[28]

Prey size selection and clearance rates of *Oikopleura fusiformis* were investigated at two sites along the northern and western shores of O'ahu, Hawai'i, during summer of 2001. Experiments were conducted to determine clearance rates of *O. fusiformis* on *Synecho-*

[27] Department of Geology and Geophysics, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Michael Bevis.

[28] Department of Oceanography, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Michael R. Landry.

coccus spp., *Prochlorococcus* spp., autotrophic eukaryotes, and heterotrophic bacteria. At each of the experimental sites, individual appendicularians were captured gently in situ in 265-ml polycarbonate bottles approximately 20 m offshore. Incubations were conducted onshore in seawater at ambient temperatures for a period ranging from 30 min to 2 hr, until the animal no longer maintained a constant feeding current. Clearance rates were determined by measuring rate of cell decline over the incubation period using flow cytometry. Average abundances of *Synechococcus* spp., *Prochlorococcus* spp., autotrophic eukaryotes, and heterotrophic bacteria during the experiments were 4×10^3 cells ml^{-1}, 2×10^4 cells ml^{-1}, 2×10^3 cells ml^{-1}, and 1×10^6 cells ml^{-1}, respectively. Average clearance rate of *O. fusiformis* was 1.4 liters $animal^{-1}day^{-1}$, ranging from 0.5 to 2.7 liters $animal^{-1}day^{-1}$, and was related to size of the organism. Clearance rates were highest on autotrophic eukaryotes (>1.0 μm) and lowest on heterotrophic bacteria (0.3–0.5 μm). These results suggest that although *O. fusiformis* feeds on less than 1.0-μm cells, they are not an efficient link between bacteria and higher consumers in the coastal subtropical ocean.

Daytime Habitat Use Patterns and the Specter of Competition for Space among Demersal Reef Fishes

Brett D. Schumacher[29]

It has been suggested that the introduced snapper *Lutjanus kasmira* (Family Lutjanidae) may compete with native reef fishes, such as the goatfishes (Family Mullidae), for dietary or spatial resources. As part of a larger investigation into the potential competitive impact this alien might have in the nearshore waters of Hawai'i, I studied the daytime spatial patterns of *L. kasmira* and several goatfishes. I conducted surveys of habitat usage patterns of these fishes on fixed transects established both on and off reef, over soft and hard bottoms, and at different depths. Although abundance often varied within species as depth and substrate changed, vertical and horizontal patterns of distribution generally did not. Water currents appeared to be an important factor affecting patterns of fish distribution and abundance. Habitat use patterns of *L. kasmira* were most similar to those of *Mulloidichthys vanicolensis*. Where comparisons between transects of different depths were possible, both species were relatively abundant at deeper sites, but were rare at shallower sites. At all sites where they occurred, both species were observed primarily low in the water column and near areas of vertical relief, though *L. kasmira* appeared to be more closely associated with the substrate. Thus, *L. kasmira* could displace *M. vanicolensis* higher into the water column, where they may be more vulnerable to predators and fishers.

CcMD1, a Novel Gene Containing a BTB/POZ Domain Sequence from the Genome of Mediterranean Fruit Fly

Pia Untalan[30]

Differentially expressed genes can be found in a variety of key developmental pathways within an organism. These pathways include the determination of sex and formation of morphological characteristics. In an effort to identify genes that are differentially expressed

[29] Hawai'i Cooperative Fishery Research Unit, Department of Zoology, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: James Parrish.

[30] Department of Cell and Molecular Biology, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: David Haymer.

during development in the Mediterranean fruit fly (medfly), *Ceratitis capitata*, a suppression subtractive hybridization (SSH) approach was initiated at embryonic and adult stages of development. An embryonic subtraction product, designated *CcMD1*, was recovered. Its cDNA sequence is 1576 nucleotides long and encodes a protein of 278 amino acid residues. Using RT-PCR, it was established that the *CcMD1* transcript is expressed in both females and males at the embryonic stage; it is also expressed in adult female abdomens, but only weakly expressed in the adult female thorax and the adult male abdomen and thorax. Weak expression is also observed at the larval and pupal stages. The difference in expression of *CcMD1* at these stages suggests that it is developmentally regulated and may play a role in key developmental processes. Sequence pattern analysis suggests that *CcMD1* is a novel gene containing a BTB-POZ domain at its N-terminus. The BTB-POZ domain is an evolutionarily conserved domain that is found in many developmentally regulated gene products and is thought to mediate protein-protein interaction. Further characterization of the representation and function of the BTB-POZ domain in the medfly has been initiated.

A Serial Analysis of Gene Expression in Sun-Damaged Human Skin

Johann Urschitz[31]

Aging of the skin consists of two simultaneous processes: intrinsic, chronologic aging and photoaging, an environmentally induced remodeling of the dermis that arises as a result of repeated exposure of skin to sunlight. Although both intrinsic aging and photoaging share some common characteristics such as decreased procollagen expression and increased expression of several matrix metalloproteinases, photoaging is considered to be the predominant contributing factor to the prematurely aged appearance of sun-exposed skin. To study phenotypic changes in human skin associated with repeated sun exposure at the transcription level, we have undertaken a comparative serial analysis of gene expression (SAGE) of sun-damaged skin and sun-protected skin as well as sun-protected epidermis. SAGE libraries were made to mRNA isolated from human preauricular (sun-damaged) skin and postauricular (sun-protected) skin, as well as epidermal nick biopsy samples. 5330 cDNA tags from the postauricular SAGE library were sequenced and compared with cDNA sequences identified from 5105 tags analyzed from a preauricular SAGE library. Of the 4742 different tags represented in both libraries, we found 34 tags with at least a four-fold difference of tag abundance between the libraries. Among the mRNAs with altered steady-state levels in sun-damaged skin, we detected those encoding keratin 1, macrophage inhibitory factor, and calmodulin-like skin protein. In addition, a comparison of cDNA sequences identified in the SAGE libraries obtained from the epidermal biopsy samples (5257 cDNA tags) and from both full-thickness skin samples indicated that many genes with altered steady-state transcript levels upon sun exposure were expressed in epidermal keratinocytes. These results suggest a major role for the epidermis in the pathomechanism of largely dermal changes in chronically sun-exposed skin.

[31] Pacific Biomedical Research Center, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Charles D. Boyd.

Investigations on Cross-Coupling of Phenylacetylene with Aryl Chlorides

Zhaohui Wang[32]

The Pd-catalyzed cross-coupling of phenylacetylene with aryl chlorides has been investigated. Among several promoters, $ZnCl_2$ can act as a co-catalyst to convert the electron-rich, electron-neutral, and electron-deficient aryl chlorides to their corresponding products in reasonable to excellent yields.

Nitrous Oxide Production in the Eastern Tropical North Pacific and the Black Sea

Marian Westley[33]

Although the oceans are known to be a major natural source of the potent greenhouse gas nitrous oxide, the magnitude and characteristics of this source are poorly constrained. Nitrous oxide is produced in the ocean as a by-product of nitrification, the conversion of ammonium in the presence of oxygen to nitrite and nitrate, and denitrification, the reduction of nitrate and nitrite under suboxic or anoxic conditions to diatomic nitrogen. To evaluate the relative importance of these two mechanisms under a range of biogeochemical conditions, I collected water samples along a transect from the oligotrophic North Pacific near Hawai'i (22.75° N, 158° W) to the highly productive, oxygen-poor waters of the eastern tropical North Pacific near Mexico (15° N, 98° W). I collected additional water samples from the Black Sea, a body of water characterized by a stable suboxic zone overlying a permanently anoxic basin. Concentration measurements revealed a large, shallow source of nitrous oxide in the eastern tropical North Pacific, most likely due to nitrification, and an insignificant source in the Black Sea. Measurements of ^{15}N and ^{18}O in dissolved nitrous oxide support the hypothesis that nitrous oxide is produced by nitrification across the subtropical North Pacific. Similar measurements performed on Black Sea samples remain puzzling and suggest either the presence of isotopically anomalous source compounds or the existence of unique biochemical pathways.

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[32] Department of Chemistry, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Craig M. Jensen.

[33] Department of Oceanography, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Brian Popp.